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The Identification of Antibiotic-Producing Bacillus from Soil

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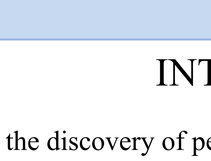
INTRODUCTION

Since the discovery of penicillin by Alexander Fleming in 1928, antibiotics have been useful tools to fight bacterial infections.[1] Antibiotics work by exploiting specific characteristics in a bacterial cell to kill it. Countless lives have been saved thanks to this. However, bacteria are particularly crafty when it comes to finding ways to 'beat' antibiotics. Their ability to rapidly mutate and adapt means that each antibiotic can only be effective for so long before it is rendered inert. In hospitals, scores of immunocompromised people are kept in very close quarters allowing for easy spread of bacteria. This gives the bacterial cells the opportunity to develop resistance to the drugs designed to kill them.

The ESKAPE pathogens are six bacteria that present the largest propensity to develop resistance and cause harm. They are *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter.*[2] They have presented a daunting issue to the medical and scientific communities. According to the Center for Disease Control, approximately 35,000 people die a year due to antibacterial resistance.[3] If we want to avoid some kind of superbug potentially killing hundreds of thousands or even millions of people, then work must be done to continue the development of new antibiotics and discover new ways to keep harmful bacteria at bay.

This is where the Tiny Earth Project steps in. It is an organization dedicated to spreading education of this issue as well as the decline in soil bacterial biodiversity. They also promote and facilitate research that students can take part in by attempting to isolate new antibiotics from the bacteria found in soil[4].



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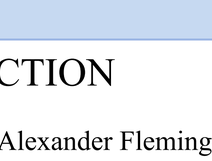

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Fig 1. Close up images of *E. coli* (left) and *B. subtilis* (right). The difference in color is a result of gram staining. A Gram- bacteria, like *E. coli,* is pink after staining due to the lack of peptidoglycan in the cell wall. Gram+ bacteria, such as *B. subtilis,* are purple because the peptidoglycan in the large cell walls retains the dye from gram staining. This can be used to help identify unknown bacteria.

We use non-threatening analogs of dangerous pathogens for this project. Two of the strains are *Bacillus subtilis* and *Escherichia coli*. *B. subtilis* is rod shaped, chain forming, gram+ bacteria that forms irregularly shaped colonies. *E. coli* is rod shaped, single or paired, gram- bacteria with round colonies.[5] While not overtly dangerous to us, these bacteria have similar morphology to the real ESKAPE pathogens. They react the same way to antibiotics and allow for testing without risking exposure to hazardous material.

The purpose of our project is to not only search for new antibiotics, but also familiarize ourselves with the practices and procedures that industry professionals use. We are on the frontlines of the battle against antibiotic resistance. There is the potential that lives could be saved with what we learn from this project.

METHODS

The bacterial strains and protocols used in this study were provided by the Tiny Earth Project Initiative (TEPI)[4]

- Soil sample collected
- Bacteria grown from soil and specific colonies isolated
- Isolates tested for antibacterial properties
- Antibiotic producing isolates further cultured and retested against ESKAPE analogs
- PCR run and DNA isolated from sample (did not work)
- Biochemical tests run to help further identify unknown bacteria

RESULTS

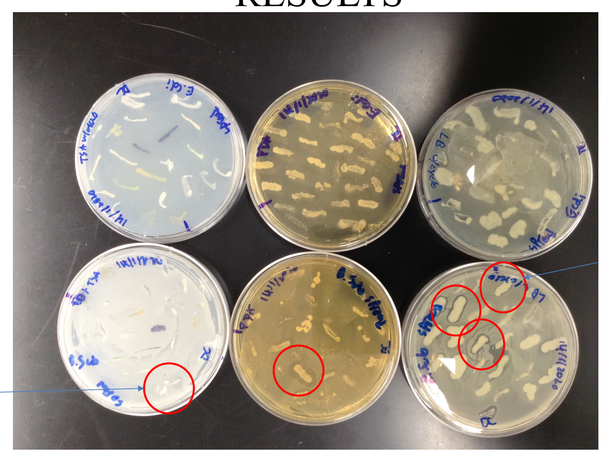


Fig. 2 Original plates of soil isolates tested against ESKAPE analogs. **Top row:** isolates tested against *E. coli*. **Bottom row:** isolates tested against *B. subtilis.* **Left column:** 10% TSA medium. **Middle column:** PDA media. **Right column:** LB agar media w/ cyclohexane. Isolates from master plates were picked and patched onto spread plates of the tester strains. All plates were incubated for about 24 hrs. at 28ºC. Red circles indicate evidence of 'halos' or regions of antibacterial production. Halos only formed when tested against *B. subtilis*.

A B C D E F

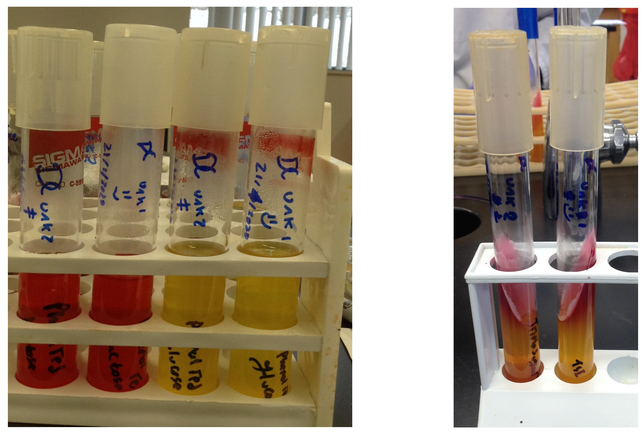


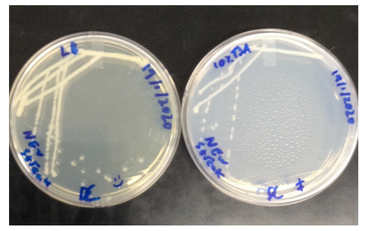
Fig. 3 Left: Phenol red indicator changed from red to yellow in the tubes containing glucose as opposed to lactose where we see no color change. There was no evidence of gas production. The results from the phenol red test indicates the bacteria metabolize glucose rather than lactose for cellular function. **Right:** In the triple sugar iron test we see red tops with bacteria growing along the surface. The bottoms of the tubes remain yellow, and we see no black which would indicate evidence of the bacteria reacting with the iron in the agar. TSI is a very useful differential indicator because there are many ways it can react depending on the type of bacteria. The two most prominent potential antibiotic producers were run through a series of biochemical tests in order to help identify them. Tubes A, C, & E contain DC-2-LB-*B.sub.* Tubes B, D, & E contain DC-22-TSA-*B.sub*. All these tests were incubated for about 24 hrs. at 28ºC.

RESULTS (CONTINUED)
Biochemical test results comparison

	DC-2-LB-*B. sub*	DC-22-TSA-*B. sub*	Streptomyces [6]	Bacillus[7]
MacConkey's agar	-	-	-	-
Simmons citrate	-	-	+	-
Blood agar	+	+	-	+
Phenol red (glucose)	+	+	-	+
Phenol red (lactose)	-	-	-	-
Triple sugar iron	+/-	+/-	-	+/-
Catalase	+	+	-	+

Results of biochemical tests on unknowns as well as *Streptomyces* and *Bacillus*. (+) indicates a positive reaction to the test, and (-) indicates a negative reaction. These tests can be used to narrow in on the identification of unknown bacteria. All tests requiring incubation were run for about 24 hrs. at 28ºC.

DC-2-LB-*B. sub*



DC-22-TSA-*B. sub*

Fig. 4 Streak plates of DC-2-LB-*B.sub* (left) and DC-22-TSA-*B.sub* (right). The morphology of the two strains isolated from the soil is very similar if not the same. We see small, ovular colonies, with a white coloration. They were grown on different media, so it cannot be said for certain that they are the same species. However, they reacted to all the biochemical tests in the same way. When compared to *Streptomyces* and *Bacillus*, they look very similar to *Bacillus*.

DISCUSSION

It was postulated that the strains pulled from the soil would most likely be *Streptomyces* or *Bacillus*. Based on the biochemical tests, the unknowns could both be *Bacillus*. *Bacillus* is a known antibiotic producer[7], so it makes sense that it would form halos when first tested. It is interesting that it only showed signs of antimicrobial activity against *B. subtilis*. Another experiment could be testing this strain against other bacteria to see if it affects their growth in the same way. Also, in order to more accurately identify the isolated bacteria, a sample of DNA should be sent in for sequencing.

LITERATURE CITED

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